REMARKS

Claims 1, 5-8, 10, 11, 13-15, 25, 26, and 28 are pending. Support for amendments to the claims is discussed below. No new matter has been added herewith. The following addresses the substance of the Office Action.

Indefiniteness

Claims 1, 5-8, 10-15 and 25-28 were rejected under 35 U.S.C. § 112, second paragraph as being indefinite. The Examiner noted that the claims were drawn to a process for amplifying TALL-104 lymphocytes comprising inoculating a multi-chamber stack with any "cell". Thus, it was unclear how inoculating any generic "cell" could result in the expansion or amplification of TALL-104 lymphocytes. Claim 1 has been amended to recite a process for amplifying TALL-104 by adding an inoculum of TALL-104 cells in a single fermentation unit, amplifying the TALL-104 cells and recovering TALL-104 cells.

Claims 13-14 were rejected as being incomplete for omitting essential elements, such omission amounting to a gap between the elements. In particular, Claim 1 was drawn to a process for amplifying TALL-104 lymphocytes in a multi-chamber stack and recovering said lymphocytes. Thus, it was unclear how the claimed methods could result in the production of frozen bags of lymphocytes without recitation of additional steps. Claim 13 has been amended to recite specific steps (a)-(c) for the preparation of frozen bags of TALL-104.

Claim 1 was found to be indefinite in the recitation of adding an inoculum of cells in an initial volume of "1/10 to 1/6 of the multi-chamber stack final volume" ... In particular, with regard to Item 8A of the Office Action, Claim 1 has been amended to recite " an inoculum of cells in an initial volume from 1/10 to 1/6 of the multi-chamber stack volume capacity, and the same volume of fresh complete medium" (i.e., the typing error of "muti"-chamber instead of "multi"-chamber has been amended, and "final volume" has been amended to "volume capacity", (as supported by the specification from page 6, line 32 to page 7, line 1). Furthermore, the phrase "and the same volume of fresh antibiotic free complete medium" has been added to clarify that equivalent amounts of inoculum and fresh antibiotic-free complete medium are added together (as supported by the specification, lines 3-4, page 7 and page 5 lines 4-6). Furthermore, Claim 1 is amended to recite "amplifying the cell number by adding a complete medium volume corresponding to the volume contained in the multi-chamber stack every 3-5 days.

Regarding Item 8B of the Office Action regarding lack of antecedent basis for "the bag" in Claim 27, Claim 27 is canceled rendering the rejection moot.

In light of the above amendments to Claim 1 and the preceding remarks, the Applicants respectfully request that the rejections of claim 1, 5-8, 10-15, and 25-28 under 35 U.S.C 112 be withdrawn.

Written Description

Claims 1, 5-8, 10-15 and 25-28 were rejected under 35 U.S.C. § 1112, first paragraph as failing to meet the Written Description requirement. With regard to point 9A, the Examiner stated that recitation of: a volume of 1/6 to 1/10 of the multi-chamber stack "final volume" had a broader scope than what is disclosed by the Specification. As discussed above, Claim 1 is amended to recite "an inoculum of cells in an initial volume from 1/10 to 1/6 of the multi-chamber stack volume capacity". Such limitation finds support on pages 6-7 of the specification where it is stated that the inoculum of TALL-104 cells is performed in a volume ranging from 1/6 to 1/10 of the cell-factory final volume capacity;

Regarding point 9B, the Examiner stated that the claims were broader in scope than what is supported by the Specification, which discloses that a volume of complete medium corresponding to that contained in the cell-factory is added every 3-5 days. Claim 1 is amended to recite "adding a complete medium volume corresponding to the volume contained in the multi-chamber stack every 3-5 days". Such limitation finds support on page 7, lines 8-10 of the Specification.

Regarding point 9C, the Examiner indicated that disclosure of a single cell concentration was not adequate to support a range of "at least" 0.75×10^6 cells as was previously claimed. Claim 6 is limited to recite "the cellular density of the inoculum is 0.75×10^6 cells/ml" by removing the phrase "at least". The limitation finds support at page 7, line 24 of the Specification as filed.

In light of the above amendments to Claims 1 and 6, the Applicants respectfully request removal of the rejection of Claims 1, 5-8, 10-15, and 25-28 under 35 U.S.C. § 112, first paragraph.

Obviousness

Claims 1, 5-8, 10-13, 15, 25-26 and 28 were rejected under 35 U.S.C. § 103(a) as being unpatentable over WO 94/26284, in view of Gambacorti-Passerini et al. (1998 *Tumori* **74**:523-530) and Tuyaerts et al. (2002 *J Immunological Methods* **264**:135-151), as evidenced by the product information for Nunc cell factories.

WO 94/26284

The Examiner states that WO 94/26284 teaches a process for amplifying TALL-104 lymphocytes by a method that comprises growing the cells in the presence of IMDM medium supplemented with 10% fetal bovine serum and IL-2, and adding fresh medium containing IL-2 on a biweekly basis, which results in the continuous growth of TALL-104 cells in a exponential fashion (i.e., adding a volume of medium "corresponding" to the initial IL-2 containing medium to amplify the cells, see page 24 and 26 in particular).

However, referring to page 6, lines 9-24 of WO 94/26284, the reference teaches a method for modifying TALL-104 cells by a short, 18-hour treatment to obtain TALL-104 cells with maximized cytotoxicity. The method disclosed by WO 94/26284 includes the steps of 1) activating the cells by treating *in vitro* with a selected cytokine, followed by 2) γ-irradiating the activated cells to irreversibly arrest their proliferation. In contrast, the presently disclosed methods relate to a process of amplifying TALL-104 cells, whereby the TALL-104 cells are amplified to reach therapeutic doses of TALL-104 cells. In contrast to the method disclosed by WO 94/26284, the recovered cells are not γ-irradiated to irreversibly arrest their proliferation. Thus, while the culture conditions of WO 94/26284 are designed to obtain an improved cytoxicity, they do not relate to amplification of cells to reach a prefixed cell number. On the other hand, the presently claimed methods significantly amplify TALL-104 cells, (i.e., starting from an inoculum of at least $0.7x10^6$ cells/ml in an initial volume from 1/10 to 1/6 of a multi-chamber stack volume capacity, and giving rise to at least $1x10^9$ TALL-104 cells). Based on the teachings of WO 94/26284, one of skill in the art would have had no reason to believe that the presently claimed methods could lead to such an amplification of TALL-104 cells.

WO 94/26284 describes the TALL-104 cell line as having been established from mononuclear cells from a leukemic sample according to O' Connor et al. *Blood* **77**:1534-1545 (1991) (see page 25, lines 13-17). Referring to page 26, lines 1-4 of the reference, the cell line

has been in continuous culture since 1990 in the laboratory of the inventor, Dr. Santoli. Regarding the origin of TALL-104 cell line, WO 94/26284 reports that mononuclear cells from leukemic sample are separated by Ficoll Hypaque gradient centrifugation and plated in Iscove's modified Dulbecco's medium (IMDM) supplemented with 10% fetal bovine serum and antibiotics (complete medium) added with IL-2 at the specific concentration (see page 25, lines 18-24). Thus, in contrast to the Examiner's interpretation, WO 94/26284 actually teaches growth of TALL-104 cells in an antibiotic containing medium. In contrast, the present application teaches and claims the use of an antibiotic-free culture medium (i.e., "complete medium" according to the definition stated at page 5, lines 4-6 of the Specification as filed). The adoption of the present homogenous culture system provides the important advantage of having a reduced contamination risk, allowing one to use an antibiotic-free culture medium. In comparison, an antibiotic-free culture medium is not possible in culture systems that use multiple flasks (see page 11, lines 11-13).

Regarding the addition of fresh media, even if WO 94/26284 teaches biweekly addition of fresh medium containing recombinant human IL-2 for optimal viability and continuous growth of TALL-104 cells, it doesn't teach adding a volume of media corresponding to the same volume contained in the cell-factory. In fact, WO 94/26284 simply teaches adding the same type of medium, not in terms of volume. As supported by the Specification at page 7, lines 8-11, Claim 1 is limited to recite "amplifying the cell number by adding a complete medium volume corresponding to the volume contained in the multi-chamber stack every 3-5 days".

Gambacorti-Passerini et al.

With reference to page 7 of the current Official Action, the Examiner states that Gambacorti-Passerini et al. teach a method for the large scale production of lymphocyte activated killer (LAK) cells comprising culturing the cells at a concentration of $1.5x10^6$ cells/ml in a 10 floor multi-chamber stack (Nunc Cell Factoriestm, see page 524 in particular). Gambacorti-Passerini et al. also teach that the killer lymphocytes can be grown in range of concentrations (2.5, 5, 10 %) of homologous human serum without affecting cell recovery (see page 525 in particular). Gambacorti-Passerini et al. also teach that the large scale production of killer cells in the multi-chamber stacks results in fully comparable activation and function of the cells compared to cells grown in standard flasks (see page 527 in particular). Gambacorti-Passerini et

al. teach that the culture method using the multi-chamber stacks is faster and more affordable than other cell culture methods (see page 529 in particular).

However, as set forth in the Applicant's previous response dated April 11, 2008, it should be noted that LAK cells are quite different from TALL-104 cells. Gambacorti-Passerini et al. used the Cell-factory to address specific technical problems unique to LAK cells. The LAK cells of Gambacorti-Passerini et al. were generated by culture of peripheral blood lymphocytes (PBL) from melanoma patients in interleukin-2 (IL-2). On the other hand, TALL-104 cells of the Applicant's methods refer to an established, immortalized T lymphocyte cell line derived from children with T-cell lymphoblastic leukemia (see page 1, line 5 of 9-12 of the specification). LAK and TALL cells possess different phenotypic characteristics, which significantly influence their clinical employment as well as their culture conditions.

Moreover, LAK cells are a heterogeneous (mixed) cell population which includes activated NK (CD3- CD16+) and T (CD3+ CD16-) cells (see page 1, lines 34-38 of WO 94/26284). Such heterogeneous lymphocyte cell populations are obtained by repeated leukaphereses from a patient (as taught by Gambacorti-Passerini et al., see in particular page 524). Depending on patient characteristics (gender, age, physical conditions), variances in cellular behavior such as activation, cell division and aggregation leading to LAK production would result in variability in the final number of cells and their vitality. Furthermore, the peripheral blood lymphocytes (PBL) of the patients must be cleaned of erythrocyte contamination, which could negatively affect LAK cell activation and vitality (Gambacorti-Passerini et al., see in particular page 523-524). In contrast, TALL-104 lymphocytes of the current invention are an established conserved and immortal cell line (see page 1, line 9-12 of the specification), which has been grown in continuous cell culture since 1990 in the laboratory of the inventor, Dr. Santoli.

LAK cells are used in adoptive immunotherapy of cancer (as taught also by Gambacorti-Passerini et al.) with several limitations in comparison to TALL-104 cells. For example, LAK cells must be derived from the same patient to whom they will be transferred back for treatment. On the other hand, TALL-104 cells can be administered to any patient, independent of histocompatibility antigens phenotype (see page 1, lines 17-21 of the Specification). For this reason, TALL-104 cells can be used in adoptive transfer immunotherapy in allogenic (HLA-

mismatched) recipients. Furthermore, LAK cells have inadequate tumoricidal activity. Thus, after *in vivo* administration of LAK cells, it is necessary to provide a concomitant treatment with cytokines (principally with IL-2), which cause significant toxicity adverse reactions.

The above mentioned differences between LAK and TALL cells illustrate that different and specialized management of the two cell types is necessary, both *in vitro* and *in vivo*. Thus, the teachings of Gambacorti-Passerini et al. regarding LAK are not applicable to the amplification of TALL-104 cells.

With regard to the "culture process", it should be noted that Gambacorti-Passerini et al. teach a process for the large scale cytotoxic activation of PBL cells to LAK cells, rather than a process to ensure a proper amplification of LAK cell number (see also Gambacoti-Passerini et al., page 529 – Discussion's chapter, lines 1-3: "In this report a new method for activation large quantities of patient's PBL for adoptive immunotherapy is described"). Thus, one of skill in the art would not have had reason to believe that the methods of Gambacoti-Passerini et al. could lead to the presently claimed amplification of at least $1x10^9$ TALL-104 cells.

Tuyaerts et al.

The Examiner asserts that Tuyaerts et al. teach that cytokine dependent cells can be grown in multi-chamber stacks (Nunc Cell-Factories) by adding the cells in an initial volume of 160 mls of cytokine containing medium per chamber, followed by supplementing the cells with complete medium containing cytokines every 48 hours after the initiation of the culture (i.e., a volume of medium "corresponding" to the medium in the multi-chamber stack.

Firstly, Tuyaerts et al. teach a method for large scale production of dentritic cells (DC) starting from the amplification of monocytes (typical adherent cells) in a Cell-factory (see Tuyaerts et al., page 136, column 1, lines 38-41), and inducing the same monocytes to differentiate into DC by means of GM-CSF in combination with IL-4 (see Tuyaerts et al., page 136, column 1, lines 29-31 and page 138, lines 33-36). Thus, Tuyaerts et al. teach the amplification of typical adherent cells, such as monocytes, by means of a typical cell culture system for adherent cells, such as the Cell—factory™. In comparison, the Applicant's disclosure relates to the amplification of TALL-104 cells, which usually grow in suspension, by means of a Cell-factory™, which is normally used for anchorage-dependent cells (see page 3, lines 21 and lines 28-30 of the Specification as filed).

Moreover, Tuyaerts et al. teach that $800x10^6$ PBMC were plated in a single tray Cell-factory, were left to adhere for 2 h, and subsequently were "cleaned" from the non-adherent cells. The adherent cells were cultured in 160 ml (SCF) medium supplemented with 1000 U/ml GM-CSF and 100 U/ml IL-4 (see Tuyaerts et al., page 138, column 1, lines 28-36).

On the other hand, the Applicant's disclosure teaches that the inoculum into a Cell-factory is performed with a number of cells ranging from 1.5 to 2.5 $x10^7$/chamber in a volume ranging from 1/6 to 1/10, preferably 1/8, of the Cell-factory final volume capacity, wherein, in the case of a 10 chamber Cell-factory the final volume capacity is stated as 2 L (see page 7, lines 1-3 of the Specification). Thus, in the case of a 10-chamber cell-factory, the Applicant's disclosure teaches an initial volume of medium from 200 to 330 ml, i.e., from 20 to 33 ml per chamber, rather than 160 ml/chamber, as reported by Tuyaerts et al.

With reference to the Examiner's calculations from the Nunc Product Information Sheet, the Examiner's calculations were not performed correctly. According to the Nunc product information sheet, the 10-chamber Cell-factory dimensions are 335mm x 205mm x 190mm. The volume, as calculated by the Examiner (335mm x 205mm x 190mm = approx. 13 L) considers the dimensions as if they were totally employable to seed cells. This is not correct since the dimensions refer to the external overall dimensions of the Cell-factory™, not to the effective culture spaces of the same. The Cell-factory™ is composed of several chambers, which are placed one above the other. Each of the chambers occupies physical space, which should not be calculated into the culture volume capacity of the Cell-factory™. The culture area published on the Nunc product information sheet is 6320 cm^2. Thus, even if one uses the value of 6320 cm^2, an inoculum of 1/6 to 1/10 of the multi-chamber stack volume capacity would be equivalent to 632-1053 ml, or 63.2-105 ml per chamber, which is different from 160 ml/chamber, as reported by Tuyaerts et al.

Furthermore, while Tuyaerts et al. teach subsequent additions of volumes of medium such as the medium of the inoculum (see Tuyaerts et al., page 138, lines 36-38), the reference only teaches adding the same type of medium. In contrast, Applicant's disclosure teaches adding the same volume of medium (see page 7, lines 2-4 and lines 8-11 of the specification and Example 3 at page 16, lines 5-8, wherein it is showed that " a cell suspension (250 ml) containing approx. $0.8x10^6$ cells/ml (i.e. $2x10^8$ cells/chamber) were inoculated into a 10 chamber cell-factory, the

same amount of complete medium was immediately added and four days later, complete medium was added (500 ml x3 times) up to a final volume of 2 L).

In conclusion, none of the cited references adopt a multi-chamber stack for amplifying TALL-104 cells. Moreover, none of the cited references provide any reason to believe that the presently claimed methods could give rise to at least $1x10^9$ TALL-104 cells in a homogeneous culture system. In view of the amendments to the claims and the preceding remarks, the Applicants respectfully request removal of the rejection under 35 U.S.C. § 103(a).

Claims 14 and 27 were rejected under 35 U.S.C. § 103(a) as being unpatentable over WO 94/26284, Gambacorti-Passerini et al. and Tuyaerts et al., in further view of U.S. Patent No. 6,491,678. In particular, the '678 patent teaches a sample chamber that can comprise up to 1 ml. However, in light of the remarks above, Claim 14 is not obvious since Claim 13 is not obvious over WO 94/26284, Gambacorti-Passerini et al. and/or Tuyaerts et al. Claim 27 is canceled without prejudice. Accordingly, the Applicants respectfully request removal of the rejection.

No Disclaimers or Disavowals

Although the present communication may include alterations to the application or claims, or characterizations of claim scope or referenced art, Applicant is not conceding in this application that previously pending claims are not patentable over the cited references. Rather, any alterations or characterizations are being made to facilitate expeditious prosecution of this application. Applicant reserves the right to pursue at a later date any previously pending or other broader or narrower claims that capture any subject matter supported by the present disclosure, including subject matter found to be specifically disclaimed herein or by any prior prosecution. Accordingly, reviewers of this or any parent, child or related prosecution history shall not reasonably infer that Applicant has made any disclaimers or disavowals of any subject matter supported by the present application.

CONCLUSION

In view of Applicants' amendments to the Claims and the foregoing Remarks, it is respectfully submitted that the present application is in condition for allowance. Should the Examiner have any remaining concerns which might prevent the prompt allowance of the

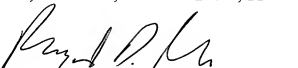
application, the Examiner is respectfully invited to contact the undersigned at the telephone number appearing below.

Please charge any additional fees, including any fees for additional extension of time, or credit overpayment to Deposit Account No. 11-1410.

Respectfully submitted,

KNOBBE, MARTENS, OLSON & BEAR, LLP

Dated: November 12, 2008

By: 

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